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Crystal Structure of Human gamma-Glutamyl Hydrolase

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Introduction: gamma-Glutamyl hydrolase catalyzes the cleavage of the gamma-glutamyl chain of folylpoly-gamma-glutamyl substrates. The enzyme plays a major role in folate and antifolyl poly-gamma-glutamate metabolism, regulating the intracellular retention of the polyglutamyl substrates.

Methods and Materials: Human gamma-glutamyl hydrolase (hGH) was crystallized in space group $P2_12_12_1$ with cell dimensions of a = 58.8 Å, b = 156.9 Å, c = 161.8 Å and with four molecules in the asymmetric unit. Crystals of hGH were soaked in a 10 mM solution of p-chloromercury benzoylsulfonic acid, a known inhibitor of the enzyme. The crystal structure was determined by MAD phasing using the anomalous signal of the mercury atom. Data, to 2.0 Å resolution, were measured at 4 wavelengths: at the inflection point, two near the absorption peak and one remote. The Patterson maps revealed the presence of 8 mercury atoms per asymmetric unit. The structure was determined using the SOLVE, MLPHARE, ARP-WARP and CNS packages. Native data were measured to 1.6 Å resolution. Final R- and R_{free}-values are 0.182 and 0.202.

Results: The asymmetric unit contains two homodimers of hGH. The overall structure contains 10 alpha-helices and 14 beta-strands. The central feature of the molecule is an eight-stranded sheet flanked by 3 and 5 helices on its sides. This fold is similar to those of class I glutamine amidotransferases. The active site contains the catalytic triad, His, Cys, Glu and is adjacent to a hydrophobic pocket that may serve as the binding pocket for the pteroyl group.